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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

To identify prostate cancer susceptibility genes, we applied a mutation screening of candidate gene approach. We screened for mutations in *CHEK2*, the upstream regulator of p53 in the DNA-damage-signaling pathway, in DNA samples isolated from 178 clinic tumors, 149 familial prostate cancer (HPC) families, 400 sporadic cases, and 423 unaffected men as control. A total of 28 (4.8%) germline *CHEK2* mutations were found among 578 patients and additional 11 in 9 families. Sixteen of 18 unique *CHEK2* mutations identified in this study were not detected among 423 unaffected men, suggesting a pathological effect of *CHEK2* mutations in prostate cancer development. Analyses of the two frameshift mutations in Epstein Barr virus-transformed cell lines using RT-PCR and Western blot analysis, revealed abnormal splicing of one mutation and dramatic reduction of CHEK protein levels in both cases. Overall. Our data suggest that mutations in CHEK2 may contribute to prostate cancer risk and that the DNA-damage-signaling pathway may play an important role in the development prostate cancer.

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Annual report
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***CHK2*, A Candidate Prostate Cancer Susceptibility Gene**
Wanguo Liu, Ph.D.
Mayo Clinic

Introduction:

Prostate cancer is the second most common cancer and the second leading cause of cancer mortality in American men. Previous studies of family history and twins with prostate cancers have shown that genetics plays a critical role in the development of this cancer. However, genetic components contributing to prostate cancer (MIM 300200) have been difficult to identify, largely due to the complexity of this disease and the presence of phenocopies in high-risk families. Regarding to the difficulties to identify high penetrant genes based on linkage analysis and positional cloning, it has been suggested that the pathogenesis of the disease is related at least, in part, to genomic mutations in multiple low-penetrant genes. Although less penetrant, such genes might play an important role at a population level. Our laboratories have recently applied a new approach to identify prostate cancer susceptibility gene(s) based on mutation screening of candidate genes involved in the DNA damage-signaling pathway and identified mutations in the ***CHK2*** gene. CHK2 is a key regulator in this pathway. It regulates a number of downstream effector proteins such as p53 and plays essential roles in coordinating DNA repair, cell cycle progression, transcriptional regulation and apoptosis in response to various DNA-damaging events. Because mutations in p53 is infrequent in prostate cancer, while common (more than 50%) in all other cancers, we **hypothesize** that CHK2, the upstream regulator of p53, could be a candidate prostate cancer susceptibility gene. Therefore, we **propose** to: 1) screen for *CHK2* mutations in 163 familial prostate cancer families, collected at the Mayo Clinic, and determine whether the mutations segregate with prostate cancer in families; 2) perform functional analyses to determine the impact of mutant CHK2 in the DNA damage-signaling pathway using a kinase activity assay; and 3) perform loss of heterozygosity (LOH) studies to determine if CHK2 functions as a tumor suppressor in prostate cancer. These results will advance our understanding of the etiology of prostate cancer and may also enable us to develop diagnostic tools for the early detection and prevention of prostate cancer.

Body:

The tasks, which we proposed to fulfil in year one and the accomplishments associated with each task, are summarized below:

Task 1. PCR amplification (**Months 0-1**)
The DNAs to be used for this study have already been isolated and aliquoted for other ongoing studies such as linkage analysis. We, therefore, can start this project right away. We will PCR amplify all 14 exons of the CHK2 gene in all of the samples including 2 affected men for each family (2 x 163 = 326). This work will be completed within 1 month from the start of this project.

Task 2. DHPLC analysis to detect *CHK2* alterations (**Months 2-6**)
A DHPLC system (Transgenomic Inc.) will be used for detection of *CHK2* alterations. The samples showing abnormal DHPLC profiles will be sequenced from both strands. This will take approximately 5 months. We may actually initiate this work prior to the completion of the PCR amplification

As we planned, we have finished PCR amplification and DHPLC analyses of all 14 exons of the *CHK2* gene in 298 samples from 149 families (two from each family). The reason that we only analyzed 149 of 163 families is that 14 families were found later that we only have DNA from one of the affected men or they do not fit for familial prostate cancer family category. In the 298 samples, a total of 24 samples with abnormal DHPLC profiles were sequenced and 11 mutations in 9 families (Others are polymorphisms.) were identified (see **Table 1** in our manuscript in Appendix; Dong *et al.*, in press).

Task 3. Confirm all of the mutations identified in the familial cases (**Months 7-12**).

a) Repeat PCR amplification using the stock DNA as template and repeat DHPLC analysis and sequencing.

b) Total RNA from the individuals who harbor *CHK2* mutations will be isolated. RT-PCR will be performed and the amplicons will be sequenced to confirm the mutations at the transcriptional level.

c) 96 normal DNA samples will be screened to determine whether the alterations identified are truly mutations or polymorphisms. True mutations should have a higher frequency in the disease group compared to the control group.

All of the mutations identified in the familial cases were confirmed by direct sequencing of the PCR products amplified from the original stock DNAs. Since we have generated EBV-transformed cell lines from each proband of each familial prostate cancer family, we also confirmed the mutations at RNA level. In 2 families in which mutations were identified in the brothers of the probands, mutations were unable to be approved at RNA level because lacking of the cell lines. However, it is now well accepted that there is only one copy for *CHK2* exon 1-9 in human genome (5-6 copies for exon 10-14), the two samples we can not confirm their mutations at RNA level contain mutations in exon 1-9. Therefore, these two mutations identified at DNA level should be also present at RNA level.

We also finished screening for *CHK2* mutations in 2 group of unaffected men as control. The first group DNA samples were obtained from 92 men participating in an ongoing NCI prostate cancer chemoprevention trial and the second group was obtained from 331 men participating in the Rochester Epidemiology Project (Melton 1966). The details for the two groups are described in the paper (in press and cope of the manuscript is in Appendix). In the total of 421 unaffected men, we only detected 6 *CHK2* mutations (1.4%) and Sixteen of 18 unique *CHK2* mutations identified in the affected men in this study were not found in the unaffected control suggesting a pathological effect of chek2 mutations in prostate cancer development.

Task 4. Based on the above mutation data, families with *CHK2* mutations will be identified. The DNA samples from other affected and unaffected family members will be screened. DNA from some blood samples (buffy coats) or tumor blocks may need to be isolated. The particular *CHK2* mutation in each family will be analyzed to determine co-segregation in the family. This work will be done in (**Month 13-16**).

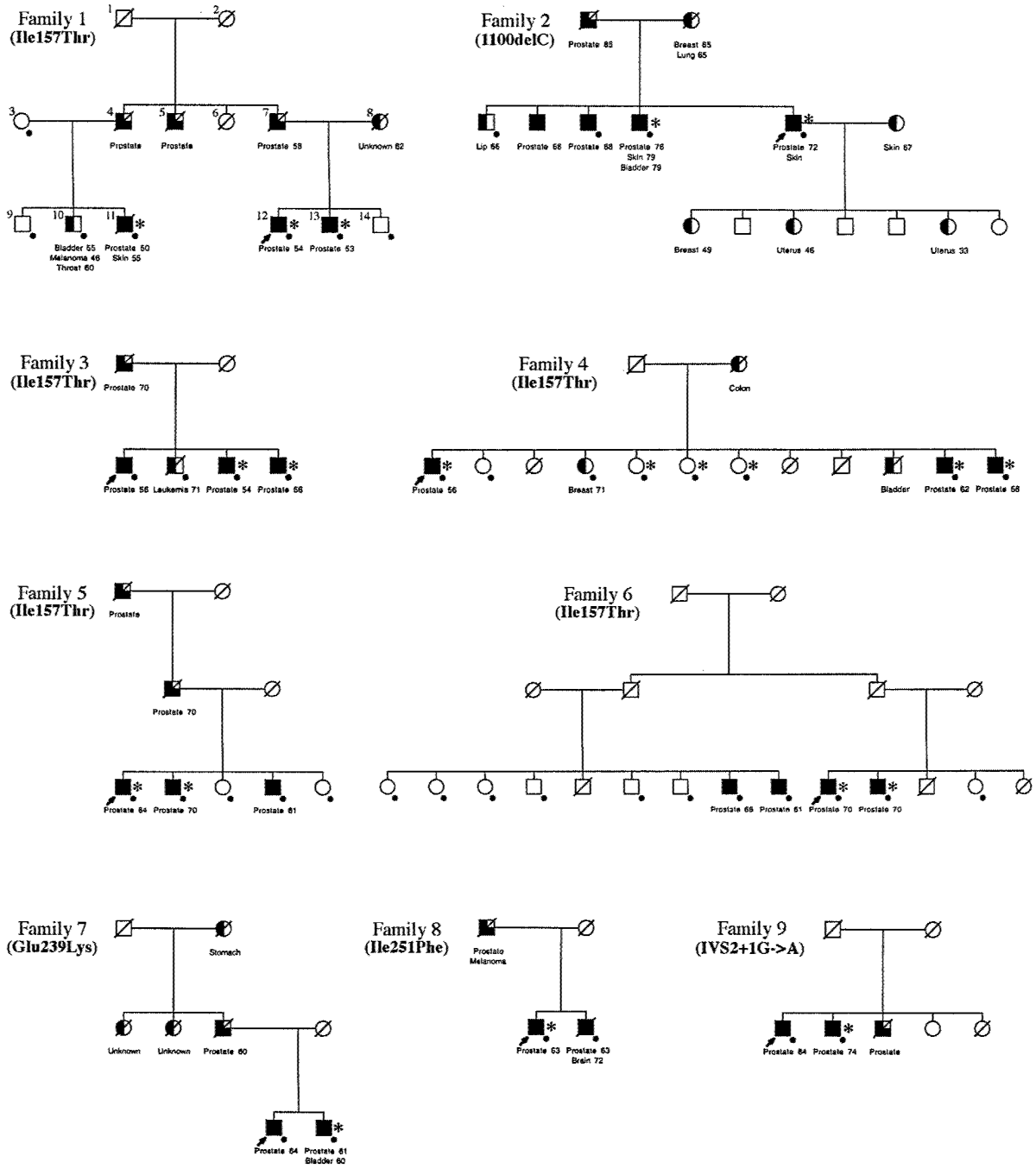
In the 9 families with *CHK2* mutations, we collected either blood or tumor samples from 45 family members and DNA were isolated and analyzed for *CHK2* mutations. The numbers of mutations identified in either affected men or unaffected family members in each family are summarized in **Table 2.**

Table 2. *CHK2* Germline Mutations in Prostate Cancer Families

Family ID	***Mutation***	***Family members***	***Aff. men***	***Aff. men with mutation/age***	***Normals with mutations***
1	Ile157Thr	7	3	3 (50, 53, 54)	0/3M + 1F
2	1100del C	4	3	2 (72, 76)	0/1M
3	Ile157Thr	4	3	2 (54, 56)	0/1M
4	Ile157Thr	8	3	3 (56, 58, 62)	3/5F
5	Ile157Thr	5	3	2 (64, 70)	0/2F
6	Ile157Thr	11	4	2 (70, 70)	0/3M + 4F
7	Glu239Lys	2	2	1 (61)	0/0
8	Ile251Phe	2	2	1 (63)	0/0
9	IVS2+1G->A	2	2	1 (74)	0/0
		45	25	17 (63.5)	0/8M, 3/12F

From 45 family members in the 9 families, we found mutations in 17 out of 25 affected men (63.5%), but not in any of the 8 unaffected men indicating that the *CHK2* mutations are only present in the men with prostate cancer. *CHK2* mutations were also identified in 3/12 females. But none of them had cancer at the time the bloods were collected. Interestingly, in the two families (#1 and 4) in which three out of three affected men harboring the *CHK2* mutations, the average ago of onset is only 55.5.

The segregation of the CHK2 mutations in 9 family is shown below in **Figure 1.**



We performed linkage analyses under the assumption of an autosomal dominant model (Smith et al. 1996) and no recombination between the underlying susceptibility locus and CHK2. Except in two families (#1 and 4), other 7 families showed evidence against cosegregation. We, therefore, could rule out only cosegregation with a highly penetrant effect but we cannot rule out a weakly penetrant effect with out data.

Key Research Accomplishments:

1. We determined that 4.8% (28/578) of the patients with prostate cancer carry *CHK2* germline mutations but only 1.4% (6/423) in unaffected men.

2. We identified *CHK2* mutations in 6% (9/149) of familial prostate cancer families and analyzed segregation of the mutations in these families.

3. We analyzed two *CHK2* mutations in two cell lines resulted in either abnormal splice or/and reduction of CHK2 protein.

Reportable outcomes: Mutations in CHK2 associated with prostate cancer risk

Conclusions: As we speculated based on our preliminary study, the *CHK2* germline mutations are present in both familial and sporadic prostate cancers but with very low frequency in unaffected men, suggesting that mutations in the *CHK2* gene is associated with prostate cancer risk. Further functional analysis of the CHK2 mutants and studies of the *CHK2* mutations in prostate cancer patients in other population will provide insight into the understanding of the susceptibility of *CHK2* and its associated the DNA-damage-signaling pathway in prostate cancer development.

References:

1. Mel LJ 3rd (1996) History of the Rochester Epidemiology Project. Mayo Clin Proc 71:266-274.

Appendices:

1. Proof of our manuscript entitled "Mutations in *CHEK2* Associated with Prostate Cancer Risk" by Dong et al., which are in press in American Journal of Human Genetics. (CHK2 has been named as CHEK2 by HUGO)

2. Abstract for the presentation at the 52nd ASHG annual meeting in Baltimore, October 15-19, 2002.

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Mutations in *CHEK2* Associated with Prostate Cancer Risk

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q1 **The DNA-damage–signaling pathway has been implicated in all human cancers. However, the genetic defects and the mechanisms of this pathway in prostate carcinogenesis remain poorly understood. In this study, we analyzed** q2 **CHEK2, the upstream regulator of p53 in the DNA-damage–signaling pathway, in several groups of patients with prostate cancer. A total of 28 (4.8%) germline *CHEK2* mutations (16 of which were unique) were found among 578 patients. Additional screening for *CHEK2* mutations in 149 families with familial prostate cancer revealed 11 mutations (5 unique) in nine families. These mutations included two frameshift and three missense mutations. Importantly, 16 of 18 unique *CHEK2* mutations identified in both sporadic and familial cases were not detected among 423 unaffected men, suggesting a pathological effect of *CHEK2* mutations in prostate cancer development. Analyses of the two frameshift mutations in Epstein Barr virus–transformed cell lines, using reverse-transcriptase polymerase chain reaction and western blot analysis, revealed abnormal splicing for one mutation and dramatic reduction of CHEK2 protein levels in both cases. Overall, our data suggest that mutations in *CHEK2* may contribute to prostate cancer risk and that the DNA-damage–signaling pathway may play an important role in the development of prostate cancer.**

Introduction

Genetic components contributing to prostate cancer (MIM 300200) have been difficult to identify, largely because of the complexity of this disease and the presence of phenocopies in high-risk families. Current genetic studies, using linkage analysis of "high-risk families" followed by positional cloning approaches, have identified more than six susceptibility loci (Ostrander and Stanford 2000). Only two studies have shown any success with the cloning of candidate susceptibility genes from these regions: *HPC1* (MIM 601518) and *HPC2/ELAC2* (MIM 605367), localized to chromosomes 1q and 17p, respectively (Tavtigian et al. 2001; Carpten et al. 2002). However, follow-up studies for *HPC2/ELAC2* have failed to replicate the original findings (Wang et al. 2001; Xu et al. 2001) or have suggested only a limited role in hereditary prostate cancer (Rebbeck et al. 2000; Wang et al. 2001). Since prostate cancer is heterogeneous in nature, and because of the difficulty in identifying highly penetrant susceptibility genes, it may be that the pathogenesis of the disease is related, at least in part, to genomic mutations in multiple low-penetrance genes. Although less penetrant, such genes might play an important role at a population level.

Genomic instability is a common feature of many human cancers (Hoeijmakers 2001). The DNA-damage–signaling pathway plays a critical role in maintaining genomic stability in response to a variety of DNA-damaging events (Khanna and Jackson 2001). Disruption of this pathway has been shown to be pivotal in cancer development, since several proteins involved in this pathway (such as BRCA1 [MIM 113705], TP53 [MIM 191170], and ATM [MIM 208900]) are frequently mutated in human cancers and in several heritable cancer-prone syndromes, such as Li-Fraumeni syndrome (LFS [MIM 151623]) and ataxia telangiectasia (MIM 208900) (Malkin et al. 1990; Miki et al. 1994; Savitsky et al. 1995). Evidence that the DNA-damage–signaling pathway is also important in prostate cancer development comes from several studies. Adenovirus-mediated antisense *ATM* gene transfer has been shown to sensitize prostate cancer cells to radiation (Fan et al. 2000), and mutation in p53 is associated with amplification of the androgen receptor (MIM 313700) gene in prostate cancer (Koivisto and Rantala 1999). In

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Table 1

***CHK2* Germline Mutations Identified in Men with Prostate Cancer and in Unaffected Control Individuals**

Number	Mutation	Amino Acid Change	Exon	Domain	Clinic Tumors 1 (n = 84)[a]	Clinic Tumors 2 (n = 94)[b]	Individuals with Sporadic Prostate Cancer (n = 400)[c]	Individuals with Familial Prostate Cancer (n = 298)[d]	Unaffected Men (n = 423)[e]
1	G190A	Glu64Lys	1	S/TQ-rich	1	0	1	0	0
2	245del15 bp	del DQEPE	1	S/TQ-rich	0	0	1	0	0
3	G434C	Arg145Pro	2	FHA	0	0	1	0	0
4	IVS2+1G→A	Frameshift	2	FHA	0	0	0	1	0
5	T470C	Ile157Thr	3	FHA	1	0	6	7	5
6	G499A	Gly167Arg	3	FHA	0	0	1	0	0
7	C538T	Arg180Cys	3	Unknown	0	2	0	0	1
8	G539A	Arg180His	3	Unknown	0	0	1	0	0
9	C541T	Arg181Cys	3	Unknown	1	0	0	0	0
10	G542A	Arg181His	3	Unknown	0	0	1	0	0
11	G715T	Glu239Stop	5	Kinase	0	0	1	0	0
12	G715A	Glu239Lys	5	Kinase	1	0	0	1	0
13	A751T	Ile251Phe	5	Kinase	0	0	0	1	0
14	G954A	Arg318His	8	Kinase	0	1	0	0	0
15	A967C	Thr323Pro	8	Kinase	1	0	0	0	0
16	A980G	Tyr327Cys	8	Kinase	0	0	1	0	0
17	1100delC	Frameshift	10	Kinase	3	1	1	1	0
18	C1427A	Thr476Lys	12	Kinase	1	0	0	0	0
Total					9 (10.7%)	4 (4.3%)	15 (3.75%)	11 (3.7%)	6 (1.4%)
P value (Ile157Thr included)[f]					<.0001	.07	.03	.08	
P value (Ile157Thr excluded)[g]					<.0001	.0003	.008	.11	

[a] Unselected prostate-cancer tumor samples collected in 1997 and 1998.

[b] Prostate-cancer tumor samples, with a younger age at onset (age <59 years), collected in 2000 and 2001.

[c] Blood samples from patients without a family history of prostate cancer.

[d] Two affected men from each of 149 families were screened.

[e] Population-based control group (n = 331) with a mean age at diagnosis of 53.4 years (range 42–83 years), an average PSA value of 0.9 (range 0.15–9.1), and normal TRUS and DRE results, plus a group of unaffected men (n = 92) enrolled in an ongoing NCI prostate cancer chemoprevention trail, who were free of clinically evident prostate cancer as assessed by DRE and PSA (<3).

[f] P values comparing each group with controls, using Armitage's test for trend. Control data: 6 (1.4%) with mutation, 417 (98.6%) with no mutation.

[g] P values comparing each group with controls, using Armitage's test for trend. Control data: 1 (0.2%) with mutation, 422 (99.8%) with no mutation.

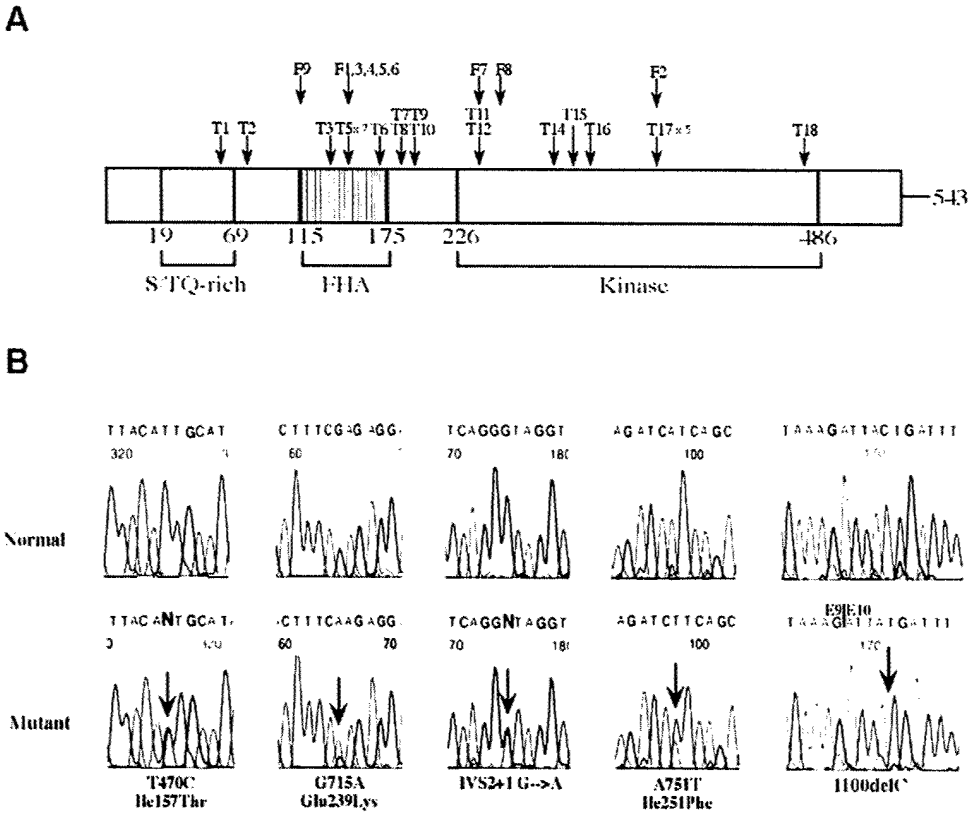

Figure 1 *CHEK2* germline mutations in prostate cancers. *A*, Mutations found in the *CHEK2* gene. "T" indicates the clinic or sporadic prostate tumor samples (numbers as shown in table 1), and "F" indicates families with prostate cancer in which *CHEK2* mutations were identified (numbers as indicated in fig. 2). *B*, Sequence analysis shows the five *CHEK2* germline mutations identified in families with familial prostate cancer. DNA sequence analyses were performed on either genomic DNA (*first four pairs of panels*) or cDNA (*right-most panels*). Sequences are presented in the 5′→3′ direction, and arrows mark the location of each mutation. The upper panels depict the regions from wild-type alleles and the lower panels show the respective sequences with the mutations. All mutations were detected with genomic DNA and were confirmed with cDNA.

addition, a low frequency of germline mutations in the breast cancer predisposition genes *BRCA1* and *BRCA2* (MIM 600185) has been identified in familial prostate cancer (Gayther et al. 2000). Moreover, the male mutation carriers in these families had been shown to have a 3.3-fold increased risk for prostate cancer, relative to the general population (Ford et al. 1994). Cumulatively, these data support the notion that the integrity of the DNA-damage–signaling pathway is essential for the prevention of prostate cancer. Since mutations in TP53, the key regulator of the DNA-damage–signaling pathway, are infrequent in prostate cancer but common in all other cancer types, we hypothesized that other components in this pathway could be mutation targets in prostate cancer.

CHEK2 (MIM 604373) is a mammalian homologue of the *Saccharomyces cerevisiae* Rad53 and *Schizosaccharomyces pombe* Cds1, both of which are involved in the DNA-damage–signaling pathway (Paulovich and Hartwell 1995; Sanchez et al. 1996; Boddy et al. 1998). CHEK2 is phosphorylated in response to various DNA-damage agents in an ATM-dependent fashion (Matsuoka et al. 1998). Activated CHEK2, along with other DNA-damage–activated protein kinases, stabilizes TP53 or enhances degradation of Cdc25A (MIM 116974) in the cell-cycle checkpoint control (Matsuoka et al. 1998; Hirao et al. 2000; Falck et al. 2001), through coordination of DNA repair, cell-cycle progression, and apoptosis (Caspari 2000; Bulavin et al. 2001). Recently, heterozygous germline mutations in the *CHEK2* gene have been identified in patients with LFS, a highly penetrant familial cancer phenotype usually

associated with inherited mutations in TP53 (Bell et al. 1999). One of the *CHEK2* germline mutations (1100delC) identified in LFS was also identified in 5.1% of noncarriers of *BRCA1* or *BRCA2* mutations in families with breast cancer, suggesting its involvement in familial breast cancer, as well (Meijers-Heijboer et al. 2002). Subsequently, somatic *CHEK2* mutations were also found in a subset of the primary tumors of LFS, such as sarcoma, breast cancer, and brain tumors, but were rare in other tumors (Allinen et al. 2001; Miller et al. 2002).

In this study, we examined DNA from patients with both sporadic and familial prostate cancers for mutations in *CHEK2*. We compared the frequency of the *CHEK2* mutations in these two prostate-cancer groups with that in an unaffected control group, to determine whether defects in CHEK2 play a role in the development of prostate cancer.

Material and Methods

Ascertainment of Patients with Prostate Cancer

Tissue.—Two separate sets of primary prostate tumor samples were collected at the Mayo Clinic and used in this study. The first set of tumor tissues ($n = 84$) was unselected and was collected between 1997 and 1998. The second set ($n = 92$) was selected for young age at diagnosis (<59 years) and was collected between 2000 and 2001. For these patients, neither family history information nor blood was available.

Blood.—For a third group, blood was collected from patients with prostate cancer ($n = 400$) with no family history of prostate cancer. These patients with sporadic prostate cancer were collected at the Mayo Clinic and were selected from respondents to a family history survey who reported no family history of prostate cancer (Wang et al. 2001). They were matched by year of diagnosis, age at diagnosis, and number of brothers in the familial group, which is described below. All but 11 of these men were treated surgically for their prostate cancer.

Familial Prostate Cancer Ascertainment

Families with familial clustering of prostate cancer were ascertained as described elsewhere (Berry et al. 2000). These families have occurrence of prostate cancer in a minimum of three men over at least two generations. Blood was collected from as many family members as possible. All men with prostate cancer who contributed a blood specimen had their cancers verified by review of medical records and pathologic confirmation. One family had Hispanic ancestry, and the remainder were q3 white. Two affected members (the proband and one randomly selected affected man from the family) from each of 149 families were initially selected for mutation analysis. When mutations were identified, the other available family members were also screened for the specific mutation.

Unaffected Control Individuals

From a sampling frame of the local population, provided by the Rochester Epidemiology Project (Melton 1996), 475 men were randomly selected for a clinical urologic examination (Oesterling et al. 1993). This exam included digital rectal examination (DRE) and transrectal ultrasound (TRUS) of the prostate, abdominal ultrasound for post-void residual urine volume, serum prostate-specific antigen (PSA) and creatinine measurement, focused urologic physical examination, and cryopreservation of serum for subsequent sex-hormone assays. Any patient with an abnormal DRE, elevated serum PSA level, or suspicious lesion on TRUS was evaluated for prostatic malignancy. If the DRE and TRUS were unremarkable and the serum PSA level was elevated (>4.0 ng/ml), a sextant biopsy (three cores from each side) of the prostate was performed. An abnormal DRE or TRUS result, regardless of the serum PSA level, prompted a biopsy of the area in question. In addition, a sextant biopsy of the remaining prostate was performed. Those men who were found to be without prostate cancer on the basis of this extensive work-up, at baseline or at any of the follow-up exams through 1994, were used for the control population ($n = 372$). To make up for study attrition, the sample was augmented with random samples from individuals in the population who were subjected to an identical workup ($n = 138$), resulting in a total sample of 510 men without evidence of prostate cancer (Roberts et al. 2000). Three hundred and thirty-one of these individuals gave informed consent to participate in this particular study. The second group of normal control DNA samples was obtained from 92 men participating in an ongoing NCI prostate cancer chemoprevention trial, all of whom were free of evidence of prostate cancer at the time blood was collected, on the basis of DRE and PSA (<3). The mean age for the 86 q4 men with age data available was 65 years (range 57.6–75.9 years). These two groups of control individuals were combined for the analysis. This study was approved by the Mayo Clinic institutional review board.

Genomic PCR and Mutation Analyses

DNA and RNA isolation from blood, tumor tissues, and cell lines were performed following the manufacturer's protocol (QIAGEN). Thirteen pairs of intronic primers covering 14 exons of the *CHEK2* gene (GenBank accession number XM_009898) were designed q5 (available upon request). Primers used for amplification of exons 10–14 were particularly designed so that either

one or both primers for each set of primers had a base mismatch in the most 3′ nucleotide, compared with sequences from nonfunctional copies of *CHEK2*. The primers thus preferentially amplified the functional *CHEK2* on chromosome 22 rather than nonfunctional copies elsewhere in the genome. PCR amplification was performed in a volume of 12.5 μl containing 25 ng of genomic DNA, each primer at 0.2 μM, each dNTP at 0.2 mM, 2.0 mM $MgCl_2$, 0.5 U of *Taq* polymerase (Ampli*Taq* Gold, Perkin Elmer), and 1 × buffer provided by the manufacturer. Denaturing high-performance liquid chromatography (DHPLC) analyses and direct sequencing of the PCR products were performed as described elsewhere (Liu et al. 1997).

RT-PCR

Lymphoblastoid cell lines from the proband of each family were established on the basis of standard procedures. Lymphocytes from peripheral blood were transformed with Epstein-Barr virus (EBV) and were cultured in RPMI-1640 medium containing 10% fetal bovine serum. All transformed cells were frozen in liquid nitrogen for future use. *CHEK2* germline mutations in these cell lines were confirmed by direct sequencing of genomic DNA. The two pairs of primers used for RT-PCR analysis of the mutations are as follows: CHK2F2 (5′-AAAAGAACAGATAAATACCGAACAT-3′) and CHKR2 (5′-TCTGCCTCTCTTGCTGAACC-3′), covering the mutations T470C, G715A, and A751T; and CHK2F3 (5′-AATTGATGGAAGGGGGAGAGCTGT-3′) and CHK2R3 (5′-TAGGTGGGGGTTCCACATAAGGT-3′), covering the 1100delC mutation. For RT-PCR analysis of the abnormal splicing products in the IVS2+1G→A mutant, one pair of exonic primers covering nucleotides 367 (in exon 2) to 564 (in exon 3) were designed (forward 5′-TATTGCTTTGATGAACCACTGC-3′; reverse 5′-TTCAGAATTGTTATTCAAAGGAC-3′). RT-PCR products were cloned into pGEM-T easy vector, according to the manufacturer's protocol (Promega). The abnormal splicing products were detected by DHPLC and were then directly sequenced. q6

Western Blot Analysis

CHEK2 proteins in the cell lines with *CHEK2* mutations were analyzed by western blot analysis. In brief, total protein from each cell line was harvested, denatured in Laemnli buffer (Bio-Rad), and separated on 8% polyacrylamide gels with prestained protein Benchmark (Gibco/BRL). After being transferred onto Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech), CHEK2 protein was visualized with rabbit polyclonal anti-CHEK2 antibody raised against N-terminal residues of human CHEK2 (kindly provided by Dr. J. Chen) by the ECL Western Blotting System (Amersham Pharmacia Biotech). The mouse monoclonal anti-β-actin antibody (clone AC-15, Sigma) was used as an internal control.

Statistical Methods

The frequencies of mutation carriers were compared among different groups, through use of Armitage's test for trend. For statistical comparisons of patients with familial disease versus control subjects, a test for trend in the number of variant alleles, analogous to Armitage's test for trend in proportions (Sasieni et al. 1997) but with the appropriate variance to account for the correlated family data, was used (Slager et al. 2002). q7 q8

Results

CHEK2 *Mutation Screening*

In this study, we screened the *CHEK2* gene for mutations in several groups of men with prostate cancer. For the first two groups, only tissue (tumor and matched normal) was available for study (clinic tumors 1 and 2 in table 1). In the 178 patients with available tissue, 13 *CHEK2* mutations were identified (table 1; fig. 1). Nine (10.7%) were detected among the 84 unselected patients with prostate cancer, and 4 (4.3%) were detected among the 94 patients with early-onset cancer. These included eight different missense mutations and 1-bp deletion mutations at nucleotide 1100 (1100delC). All of the mutations altered evolutionarily conserved amino acids, with the exception of the Arg181Cys mutation in exon 3. These mutations were considered to most likely be germline mutations, since they were present in both tumor and matched normal prostate tissues. However, since DNA from blood was not available for analysis, there is a possibility that they may represent very early somatic events. In an effort to address this concern, DNA from blood leukocytes was obtained from an additional 400 patients with prostate cancer without a family history of prostate cancer. Fifteen *CHEK2* mutations were identified in this third group (3.75%) (table 1). Although there are differences in the frequency of *CHEK2* mutations among the three prostate cancer groups, the overall incidence of *CHEK2* mutations present in these patients (28/578, 4.8%) suggest that *CHEK2* germline mutations are likely to be associated with development of a subset of prostate cancer. q9

To investigate whether the *CHEK2* mutations are also present in familial prostate cancer, we screened two affected members from each of 149 families with familial prostate cancer collected at the Mayo Clinic (Berry et al. 2000). Five different *CHK2* mutations in nine families were identified (table 1; fig. 1). Three were missense mutations—one in exon 3 (T470C, Ile157Thr) and two in exon 5 (G715A, Glu239Lys and A751T, Ile251Phe). The

other two were frameshift mutations, including the 1100delC mutation and a splice-site mutation in intron 2 (IVS2+1G→A). All five mutations changed amino acids in either the FHA (forkhead homology-associated) or the kinase activation domain of CHK2, which have previously been shown to be important for protein-protein interaction and phosphorylation of p53 in DNA-damage–signaling (Durocher et al. 2000; Shieh et al. 2000; Li et al. 2002). The presence of these mutations in such important functional domains further suggested that these *CHK2* mutations could be deleterious.

To evaluate the association between the *CHEK2* mutations and prostate cancer risk, we screened a group of unaffected men ($n = 423$). This group was comprised of two individual sets (table 1). One set contained 331 population-based unaffected control men (Wang et al. q10 2001), and the other was comprised of 92 normal con- q11 trol men free of evidence of prostate cancer. Within this control group, two different mutations among six individuals were detected: Arg180Cys ($n = 1$) and Ile157Thr ($n = 5$) (table 1). For the six unaffected men with *CHEK2* alterations, there was no evidence of disease at the time of blood collection. However, the mean age of these individuals at the time of collection was only 59.6 years (range 45.5–67.0 years), much younger than 71 years, the mean age at diagnosis of prostate cancer for whites in the United States (Bell et al. 1999). Although it is possible that these individuals may develop prostate cancer or other malignancies occurring in LFS or LFS-like syndromes later in life, it is also likely that the Ile157Thr alteration represents a polymorphism rather than a causative mutation.

Among the mutations detected, the frequency of the Ile157Thr mutation did not appear to differ between case (1.6%) and control (1.18%) individuals. We therefore tested the significance of our mutation data with and without this alteration. A global test using Fisher's exact test showed a significant difference among all of the groups ($P = .002$). When the Ile157Thr mutation was omitted, the P value was <.0001. Each of the four case groups was then compared individually with the pooled control groups. With all of the data included, only the first unselected group and the sporadic case group showed a statistically significant increase in the frequency of *CHEK2* mutations, compared with the control group (table 1). When the Ile157Thr mutation was excluded, each of the three nonfamilial groups demonstrated statistically significant increases ($P < .0001$, .0003, and .008, respectively). In both analyses, the frequency of *CHEK2* mutations in the familial group was not statistically different than the control group. When the mutations are broken down into four different categories (1100delC, all truncating mutations, all missense mutations, and all missense mutations except Ile157Thr), the associations between the mutations and prostate cancer risk are still significant, with the exception of 1100delC. However, the numbers within each category are too small to allow conclusions to be drawn. The 1100delC mutation has been proposed to confer a low penetrant risk associated with breast cancer risk. Whether it is also a risk factor for prostate cancer or other cancers remains to be elucidated.

CHEK2 *Mutations Present in Families with Familial Prostate Cancer*

To determine whether *CHEK2* mutations cosegregated with prostate cancer in the nine families (families 1–9 in fig. 2), we analyzed the DNA from all available family members for *CHEK2* mutations, including both affected and unaffected individuals. Families 1, 3, 4, 5, and 6 had the Ile157Thr mutation. This alteration was present in all affected individuals in two of the five families (families 1 and 4). Family 1 had six prostate cancer cases in two generations. The Ile157Thr mutation was present in all three affected men, including two brothers (individuals 12 and 13) and their cousin (individual 11), q12 and was absent from three unaffected male siblings (individuals 9, 10, and 14). Although not tested directly, the proband's father (individual 7) and paternal uncle (individual 4) are also expected to be carriers of this mutation, since both are affected and have affected sons with a mutation. In family 4, all the individuals affected with prostate cancer carried the Ile157Thr mutation. However, three sisters also carried the mutation but had no evidence of cancer.

Four families (families 2, 7, 8, 9) had mutations other than Ile157Thr. Family 2 is a family with multiple cancers, including five prostate cancers, two breast cancers, two uterine cancers, three skin cancers, one lung cancer, one bladder cancer, and one lip cancer, in six males and five females in three generations (fig. 2). The proband harbors the 1100delC mutation. Analysis of the available DNA from three affected men and one unaffected man of this family revealed the mutation in two affected men but not in the unaffected brother. The proband's daughters, one of whom was diagnosed with breast cancer and two of whom were diagnosed with uterine cancer, were not available for study. In the other three families, the *CHEK2* mutation was detected in only one of the two affected brothers. Overall, analysis of *CHEK2* mutations in available family members from all nine families revealed that 17 of 25 (68%) affected men harbored *CHEK2* mutations, whereas none of the unaffected men ($n = 8$) carried the mutation (fig. 2).

To test for cosegregation of *CHEK2* mutations with prostate cancer, we performed linkage analyses under the assumption of an autosomal dominant model (Smith et al. 1996) and no recombination between the underlying susceptibility locus and *CHEK2*. Although seven q13

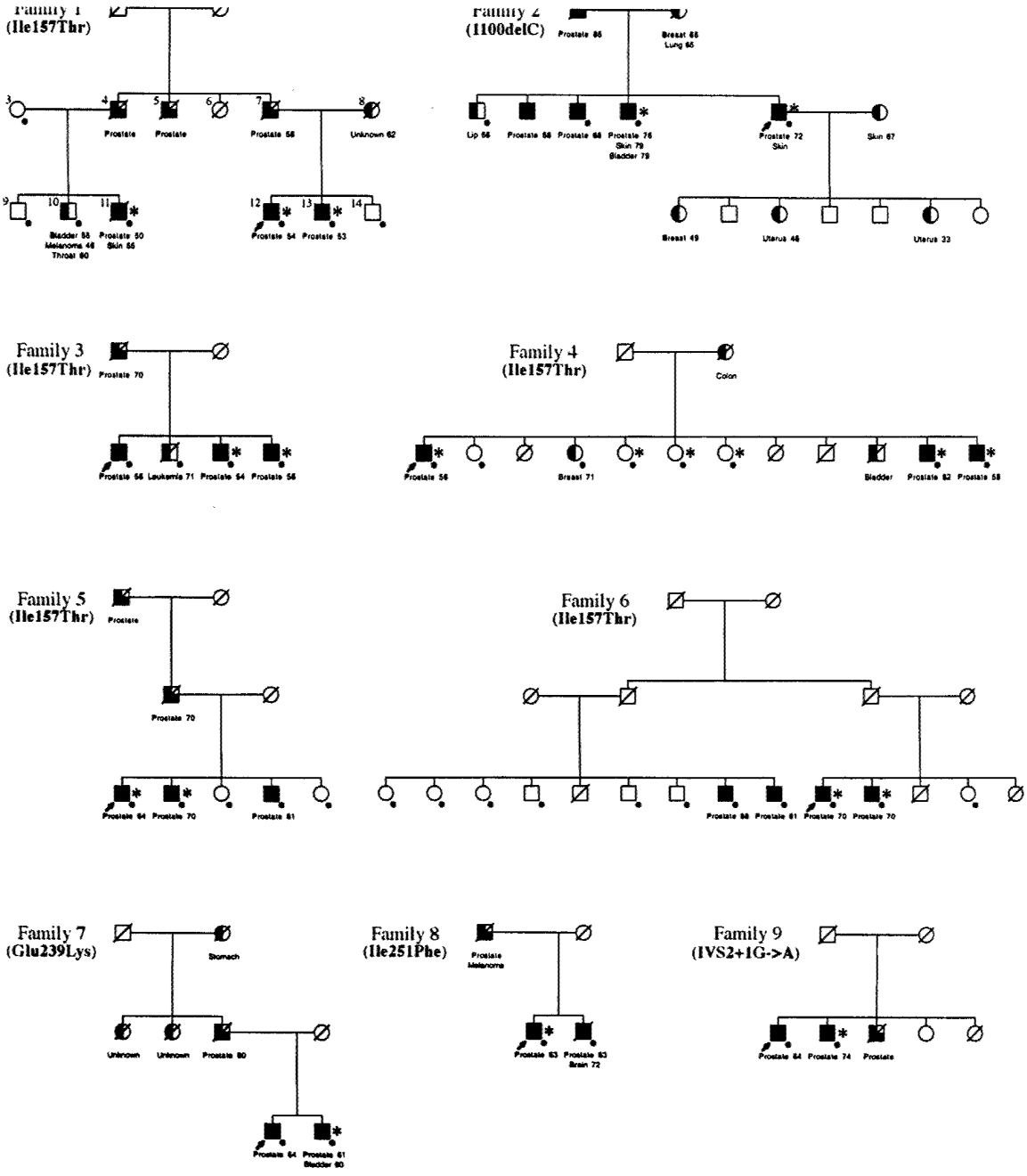

Figure 2 Segregation of *CHEK2* mutations in nine families with prostate cancer (families 1–9). Where known, the individual's age is indicated to the right side of each cancer. A dot (●) is present at the lower right corner of the symbol if a blood sample was available and was analyzed. An asterisk (*) to the right of the symbol indicates the presence of the *CHEK2* mutation carriers in each family. The individual indicated with a dot but without an asterisk has tested negative for *CHEK2* mutation. Arrows (↗) indicate probands. Squares denote males; circles denote females; completely blackened symbols denote patients with prostate cancer for whom pathology records were available; 3/4 blackened symbols denote patients with prostate cancer for whom records were unavailable; 1/2 blackened symbols denote patients with other types of cancer; all symbols with a diagonal denote deceased individuals. The cancer type for each individual is shown underneath each symbol.

of nine families showed evidence against cosegregation, we could rule out only cosegregation with a highly penetrant effect; we cannot rule out a weakly penetrant effect with our data.

Mutant CHEK2 *Altered Protein Expression*

The functional importance of the *CHEK2* mutations in prostate cancer development was explored by examination of the mutant gene products. RT-PCR analysis of the EBV-transformed cell lines that were established from the leukocytes of each proband confirmed that all *CHEK2* mutations were present in their transcripts, including the 1100delC mutation (fig. 1). The splice-site mutation (IVS2+1G→A) results in a 4-bp insertion due to an abnormal splicing using an alternative
q14 splice donor site in intron 2 (fig. 3*A* and 3*B*). This mutation creates a premature termination codon in exon 3 and eliminates part of FHA domain and the entire kinase activation domain of CHEK2. Western blot analysis of the two frameshift mutations in the patients' cell lines showed dramatic reduction of CHEK2 protein levels in both cases (fig. 3C). Reduction of CHEK2 protein has been shown to reduce the kinase activity of CHEK2 in response to DNA damage (Matsuoka et al. 2001). Altogether, our data provide evidence that some of the *CHEK2* mutations identified in the patients with prostate cancer whom we studied lead to disruption of CHEK2 expression.

Discussion

In the present study, we identified 18 unique germline *CHEK2* mutations among 28 (4.8%) individuals with prostate cancer and in nine families with familial pros-

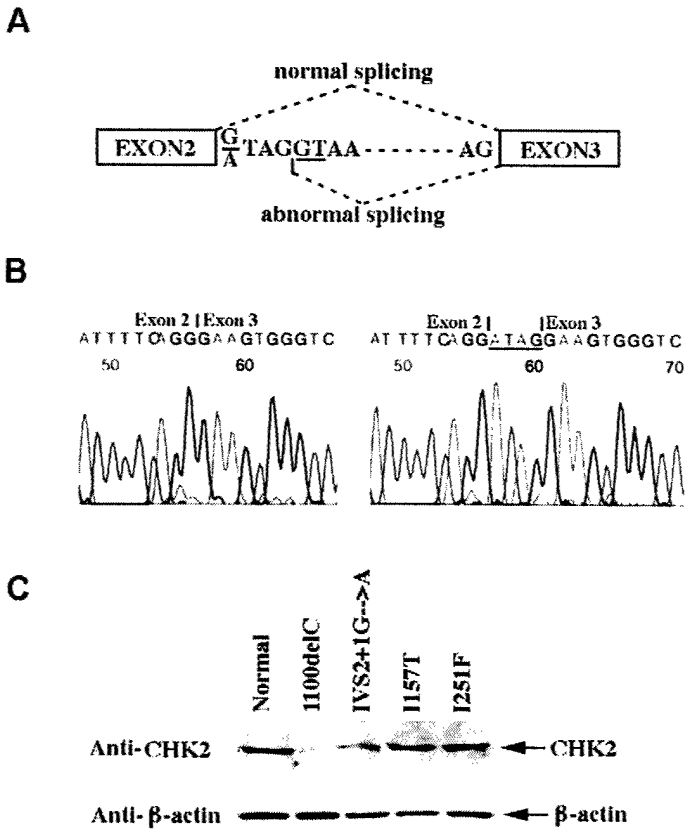

Figure 3 Abnormal splicing and abnormal protein syntheses of the two *CHEK2* frameshift mutations. *A*, Schematic representation of the abnormal splice for the IVS2+1G→A mutant. A 4-bp insertion is created in the mutant transcript because of the usage of the new splice donor site (*underlined*). *B*, Sequences of the wild-type (*left*) and mutant (*right*) *CHEK2* transcripts (between exons 2 and 3) from the cell line established from the affected men carrying the IVS2+1G→A germline mutation. C, Western blot analyses showing the reduction of CHEK2 in the cell lines carrying the frameshift mutations, compared with the normal lymphocyte cells and the cells carrying *CHEK2* missense mutations.

tate cancer. With the exception of two mutations (1100delC and Ile157Thr) that were previously reported in LFS (Bell et al. 1999), all *CHEK2* mutations identified in the present study are unique to prostate cancer. Moreover, some of the mutations presumed to be deleterious are represented by two new truncation mutations (IVS2+1G→A and Glu239Stop), which are predicted to lose their kinase activities.

Association studies between patients with sporadic disease and unaffected control individuals indicated an increased risk of developing prostate cancer in men harboring *CHEK2* mutations. The risk appears to be higher when the Ile157Thr mutation is excluded. In contrast, the frequency of *CHEK2* mutations in the familial group was not significantly different from that in the control group (table 1). Although the small sample size may account for this, the finding may reflect the presence of more highly penetrant genes in the familial group, compared with the other groups. In addition, the patients having *CHEK2* mutations in the familial cases may themselves represent phenocopies—that is, the prostate cancer in these patients may be due to *CHEK2* mutations and not due to other highly penetrant susceptibility genes segregating within the family. We recognize that well-designed epidemiological studies of large sample sets will be necessary to determine the relevance of these mutations in families with familial prostate cancer.

Germline *CHEK2* mutations were first reported by Bell et al. (1999) in patients with classic LFS and wild-type p53. LFS is a highly penetrant familial cancer syndrome, classically associated with germline mutations of *TP53*. The spectrum of cancers in this syndrome includes breast cancer, soft tissue sarcoma, brain tumors, osteosarcoma, leukemia, and adrenocortical carcinoma (Birch et al. 1984, 1990). Recently, the germline 1100delC mutation was identified in noncarriers of *BRCA1* or *BRCA2* mutations from families with breast cancer, the primary cancer in LFS (Meijers-Heijboer et al. 2002). This mutation is thought to confer a low penetrance for breast cancer. Allinen et al. (2001) screened the *CHEK2* gene in 79 Finnish families with hereditary breast cancer that did not have mutations in *BRCA1, BRCA2,* or *TP53*. However, they found only the Ile157Thr alteration, which was also present in 6.5% of control DNA samples. To date, other than somatic *CHEK2* mutations, there is no germline *CHEK2* mutation reported in other primary tumors of LFS (Miller et al. 2002). It is important to point out that there was no evidence, on the basis of published criteria, that the nine families with familial prostate cancer in which we detected germline *CHEK2* mutations had LFS or LFL syndrome (Li et al. 1988; Birch et al. 1994). Ascertainment of these families included collection of family history through telephone interviews and construction of formal pedigrees.

The most common *CHEK2* mutation identified in our study was Ile157Thr. The role of this mutation, however, is controversial, even though both genetic and biochemical data from previous studies suggest that this mutation is deleterious (Bell et al. 1999; Falck et al. 2001; Li et al. 2002). On the other hand, this mutation was found in 2.1% (2/95) of healthy population control individuals in Finland and was proposed as a polymorphism (Vahteristo et al. 2001). Other reports also indicate that this mutation is relatively common in normal healthy control individuals (Allinen et al. 2001; Meijers-Heijboer et al. 2002). In our current study, the frequency of this variant was not significantly different among the several groups of samples tested (1.21% for the sporadic prostate cancer groups, 2.34% for familial prostate cancer, 1.18% for unaffected control groups) (table 1). Whether this functional related *CHEK2* variant confers susceptibility to prostate cancer, or even to other cancers, remains to be clarified.

The presence of *CHEK2* mutations in prostate cancer highlights the importance of the integrity of the DNA-damage–signaling pathway in prostate cancer development. The fact that mutations in BRCA1 and BRCA2, two other proteins in this pathway, confer an increased risk of prostate cancer further supports this notion (Gayther et al. 2000). Moreover, the recently developed genomic instability-based transgenic mouse model for prostate cancer demonstrated the presence of a similar phenotype of early stages of human prostate cancer and that the genomic instability could be an early event in this disease (Voelkel-Johnson et al. 2000). Overall, our data provide new genetic evidence for the involvement of the DNA-damage–signaling pathway in prostate cancer development. Although the mechanism by which *CHEK2* mutations contribute to the development of prostate cancer remains unclear, future studies will add to the observations in the present report. The finding of germline mutations in *CHEK2* in both sporadic and familial prostate cancer may facilitate early diagnosis of this cancer and may provide additional insights into the biology of this malignancy, for future therapeutic applications.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

q19 GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *CHEK2* [accession number XM_009898])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for prostate cancer [MIM 300200], HPC1 [MIM 601518], HPC2/ELAC2 [MIM 605367], BRCA1 [MIM 113705], TP53 [MIM 191170], LFS [MIM 151623], ataxia telangiectasia and ATM [MIM 208900], androgen receptor [MIM 313700], BRCA2 [MIM 600185], CHEK2 [MIM 604373], and Cdc25A [MIM 116974])

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Mutations in CHK2 associated with prostate cancer risk. *X. Dong[1], L. Wang[1], K. Taniguchi[1], X. Wang[1], J.M. Cunningham[1], S.K. McDonnell[2], C. Qian[1], A.F. Marks[1], S.L. Slager[2], B.J. Peterson[2], D.I. Smith[1], J.C. Cheville[1], M.L. Blute[3], S.J. Jacobsen[2], D.J. Schaid[2], D.J. Tindall[3], S.N. Thibodear[1], W. Liu[1].* 1) Division of Experimental Pathology, Dept of Laboratory Medicine and Pathology; 2) Dept of Health Sciences Research; 3) Dept of Urology, Mayo Clinic/Mayo Medical School.

The DNA damage-signaling pathway has been implicated in the development of nearly all human cancers. However, the genetic defects and the mechanisms of this pathway in prostate carcinogenesis are poorly understood. Here we show that CHK2, the upstream regulator of p53 and the mutation target in Li-Fraumeni syndrome (LFS), is mutated in a subset of prostate cancer. Among 3 groups of sporadic prostate cancers, 13 CHK2 germ-line mutations (9 of which were unique) were identified in 262 patients. Screening for mutations in 2 affected individuals from each of 150 familial prostate cancer families revealed 9 CHK2 mutations (5 unique) in 9 families. These mutations included two frameshift and three missense mutations and segregated with prostate cancer within these pedigrees. Importantly, 9 of the 11 unique CHK2 mutations identified in the sporadic and familial cases were not detected among 423 unaffected men, suggesting a pathological effect of CHK2 mutations in prostate cancer development. Functional analyses of the two frameshift mutations in EBV-transformed cell lines, using RT-PCR and Western blot analysis, showed abnormal splicing for one mutation and dramatic reduction of CHK2 protein levels in both cases. Overall, our data indicates that mutations in CHK2 may contribute to prostate cancer risk and that the DNA damage-signaling pathway may play an important role in the development of prostate cancer.

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Use of a human expression system and microarray facilitates analysis of genome-wide alterations in MMR and APC/β-catenin -modulated colorectal cancer models. *Z. Yuan[1], T. Sotsky Kent[1], J. Trojan[2], T.K. Weber[1].* 1) Albert Einstein Col Medicine, NY, NY; 2) Johann Wolfgang Goethe University, Frankfurt, Germany.

Introduction: Pathways of malignant transformation in MMR- and APC/β-catenin-deficient colorectal cancer (CRC) await clarification. We employed cDNA microarray of a human expression system to compare gene expression profiles of CRC models of these two systems. **Methods**: MMR- and APC/β-catenin-deficient lines were engineered from a 293T human expression system with the following pathologic mutations: hMLH1-T117M, hMLH1-K618T, hMLH3-del Exon 7, APC-Δ1309, β-catenin-Δ45. MMR and APC protein expression were assayed by Western blot. MMR phenotype was assessed by functional assays and DNA sequencing. cDNA microarray was performed to compare hMLH1-MT to -WT, and APC and/or β-catenin-MT to -WT. Results were considered significant when expression ratios >1.5 were consistent in 4 of 5 experiments. Real-time PCR confirmed microarray results. **Results**: cDNA microarray identified 417 and 729 genes differentially expressed in MMR and APC/β-catenin-deficient systems, respectively. Significant expression ratios were detected in MMR-deficient lines in DNA repair, TGF-β/SMADs, apoptosis, and RAS/MAPK pathways. Oncogenes and tumor suppressor genes consistently differentially expressed in MMR-deficient lines included DLC, DOC-1, SWI/SNF2, and SWI/SNF3. In APC/β-catenin-deficient models, differential expression occurred in Wnt, cadherin, and apoptosis pathways. **Discussion**: We used a human expression system to demonstrate that, in MMR deficiency, TGF-β/SMADs signal transduction is blocked, leading to Ras/MAPK activation, and apoptosis inhibition. In the APC/β-catenin-modulated system, β-catenin levels increase due to decreased degradation, and Wnt and Cadherin signal transduction are increased. These changes promote cell proliferation, decreased mobility, and increased oncogene transcription. These data indicate cDNA microarray analysis of a controlled human expression system facilitates comparative analyses of transformation pathways in MMR- and APC-deficient CRC models. This technique provides a unique opportunity to study genome-wide alterations associated with malignant transformation in CRC.

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Haplotypes predict therapeutic ovarian cancer response. *R. Mirhashemi[1], J.F. Arena[1], N. Lambrou[1], J. Arboleda[1], M. Hunt[1], H. Averette[1], M. Penalver[1], M. Thomas[2], Z. Gaskin[2], S. Gunjupulli[2], V. Kondragunta[2], P.K. Nachimuthu[2], P. Visu[2], S. Natarajan[2], S. Gunturi[2], T. Frudakis[2].* 1) Dept OB/GYN, D-52, Univ Miami Sch Medicine, Miami, FL; 2) DNAPrint Genomics, Inc., 900 Cocoanut Ave. Sarasota, FL 34236.

Thirty five percent of Ovarian Cancer (OC) patients fail to respond to first-line combination paclitaxel (taxol) and carboplatin (TC) therapy. Poor first line chemotherapy response portends significantly higher patient morbidity and mortality. Because OC patients exhibit wide variability in the TC metabolism, it is possible that some or all of this variable response can be explained in pharmacogenetic terms. To determine whether common polymorphisms are associated with variable TC response, we applied novel analytics and data resources for a candidate gene survey of several drug metabolism genes. After genotyping 42 ovarian cancer patients at 112 SNPs in xenobiotic metabolism genes, we used an empirical (lexigraphical) approach to screen all possible SNP combinations to identify those with haplotype alleles that were genetic features of variable TC response. At the present time, we have identified two such combinations. The first is comprised of 3-SNPs spanning 9.6Kb of the 3 half of the CYP3A4 gene (avg. $p<0.004$). The second combination contained 3-SNPs spanning a 7.5Kb segment of the 3 half of the CYP2C8 gene (avg. $p<0.008$). Approximately 61% (25/41) of the patients exhibited a positive Overall TC response during first line treatment. These TC response rates are roughly comparable to the 65% average positive response rate described in previous reports for TC combination therapy. Using an overall clinical response criteria for evaluation of response over the treatment line the accuracy of the responder classification was 96%, the sensitivity of the non-responder classification was 90% and the test overall efficiency was 81%. Our results suggest that first-line TC response is largely a function of xenobiotic metabolism in OC patients, rather than tumor type or stage, and that CYP3A4 and CYP2C8 haplotype combinations are potentially useful for prescreening OC patients for an individualization of chemotherapy.

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Cox-2 gene promoter haplotypes and prostate cancer susceptibility. *R.K. Panguluri[1], S. Wang[1], W. Chen[1], W. Issacs[2], C. Ahaghotu[3], R.A. Kittles[1,3].* 1) National Human Genome Center at Howard University, Washington, DC; 2) Johns Hopkins University Oncology Center, Baltimore, MD; 3) Department of Urology, Howard University Hospital Washington, DC.

Archidonic acid metabolizing enzymes such as Cyclooxygenase-2 (Cox-2) are implicated in prostate cancer development and/or progression. The expression of Cox-2 is strongly correlated with increased tumor microvasculature. Cox-2 also plays an important role in inhibiting apoptosis, stimulating angiogenesis and promoting tumor cell metastasis and invasion. The Cox-2 promoter consists of various transcriptional regulatory elements. Polymorphisms in the 5' regulatory regions of the Cox-2 gene may have profound effects on the expression of the enzyme. To this end, approximately 1,400 nucleotides of the Cox-2 promoter region were screened using dHPLC. Four novel SNPs, -1285A/G, -1265G/A, -899G/C and -297C/G, were detected and confirmed by direct sequencing. Three clinical populations consisting of African American, Nigerian, and European American prostate cancer cases and age and ethnicity matched controls were genotyped for three of the promoter SNPs. Haplotypes were generated using the EM algorithm. Association analyses of prostate cancer and related clinical features were performed by contingency and regression analyses using single markers and haplotypes. Association analyses of single markers revealed that the -1285 and -899 SNPs were associated with prostate cancer in European Americans and Nigerians ($p<0.04$) and also aggressiveness of cancer in European Americans ($p<0.01$). Six haplotypes were observed in African American and European American cases, while only four were found in control populations. Interestingly, all three SNPs were in strong linkage disequilibrium (D'0.90; $p<0.001$) in Nigerians and African American cases but not in European American cases. One of six haplotypes was strongly associated with early onset prostate cancer (<65 years of age) (OR= 6.7; $p=0.0001$) in African Americans and Nigerians. These data suggests that SNPs in the Cox-2 promoter may influence the risk and development of prostate cancer and haplotypes may be informative for screening at-risk populations.

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Mutations in CHK2 associated with prostate cancer risk. *X. Dong[1], L. Wang[1], K. Taniguchi[1], X. Wang[1], J.M. Cunningham[1], S.K. McDonnell[2], C. Qian[1], A.F. Marks[1], S.L. Slager[2], B.J. Peterson[2], D.I. Smith[1], J.C. Cheville[1], M.L. Blute[3], S.J. Jacobsen[2], D.J. Schaid[2], D.J. Tindall[3], S.N. Thibodear[1], W. Liu[1].* 1) Division of Experimental Pathology, Dept of Laboratory Medicine and Pathology; 2) Dept of Health Sciences Research; 3) Dept of Urology, Mayo Clinic/Mayo Medical School.

The DNA damage-signaling pathway has been implicated in the development of nearly all human cancers. However, the genetic defects and the mechanisms of this pathway in prostate carcinogenesis are poorly understood. Here we show that CHK2, the upstream regulator of p53 and the mutation target in Li-Fraumeni syndrome (LFS), is mutated in a subset of prostate cancer. Among 3 groups of sporadic prostate cancers, 13 CHK2 germ-line mutations (9 of which were unique) were identified in 262 patients. Screening for mutations in 2 affected individuals from each of 150 familial prostate cancer families revealed 9 CHK2 mutations (5 unique) in 9 families. These mutations included two frameshift and three missense mutations and segregated with prostate cancer within these pedigrees. Importantly, 9 of the 11 unique CHK2 mutations identified in the sporadic and familial cases were not detected among 423 unaffected men, suggesting a pathological effect of CHK2 mutations in prostate cancer development. Functional analyses of the two frameshift mutations in EBV-transformed cell lines, using RT-PCR and Western blot analysis, showed abnormal splicing for one mutation and dramatic reduction of CHK2 protein levels in both cases. Overall, our data indicates that mutations in CHK2 may contribute to prostate cancer risk and that the DNA damage-signaling pathway may play an important role in the development of prostate cancer.